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Chemical composition and *in vitro* antioxidative potential of essential oil isolated from *Curcuma longa* L. leaves

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ABSTRACT

Objective: To determine the chemical composition and antioxidant potential of essential oil isolated from the leaves of *Curcuma longa* (turmeric). **Methods:** Chemical composition of the oil was analyzed using GC-MS. Antiperoxidative potential was evaluated using linoliec acid emulsion system. Free radical scavenging activity was evaluated using stable DPPH and ABTS free radicals. **Results:** GC-MS analyses showed that major compound present in the turmeric leaf oil is b-sesquiphellandrene (22.8%) followed by terpinolene (9.5%). Essential oil also exhibited reductive potential and antioxidant potential in linoleic acid emulsion system along with DPPH and ABTS free radical scavenging potential. **Conclusions:** The overall result suggests that turmeric leaf oil is capable of retarding oxidation reaction and free radical mediated damage and can be developed as a potent natural antioxidant.

1. Introduction

Free radicals play a crucial role in the development of tissue damage in various human diseases such as cancer, cardiovascular diseases, aging, neurodegenerative disease, arteriosclerosis and pathological events in living organisms[1,2]. Previous research reports suggest that higher intake of antioxidant rich food is associated with decreased risk of degenerative diseases particularly cardiovascular diseases and cancer[3]. An antioxidant can be defined in simple terms as anything that inhibits or prevents oxidation of a susceptible substrate. Spices and herbs are recognized as sources of natural antioxidants that include carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols and tocotrienols to prevent oxidation of the susceptible substrate[4]. The plant-based dietary antioxidants are believed to have an important role in the maintenance of human health because endogenous antioxidants provide insufficient protection against the constant and unavoidable challenge of reactive oxygen species (ROS)[5].

Since ancient times people have used spices for preventing food deterioration and pathogenic diseases. Essential oils from spices and herbs are valuable natural products used as raw materials in many fields such as perfumes, cosmetics, aromatherapy, and nutrition[6]. Essential oils have also been used in gastric discomfort, flatulence, colic and to stimulate the appetite. Apart from their use as aroma additives in food, essential oils from aromatic spice plants have a potential to be used in small amounts in fat-containing food systems to prevent or delay some chemical deteriorations occurring during the storage of these products[7]. The inhibiting and damaging effect of the oils on many life processes has been turned to our advantage in the use of these as antioxidant, bactericidal and fungicidal agents[8].

Turmeric, *Curcuma longa* L. (Zingiberaceae) rhizome, commonly used as a spice, is well known for its medicinal properties including antioxidant activity, anti-protozoal activity, anti-tumour activity, anti-inflammatory activity and

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antivenom activity [9]. The essential oil of turmeric rhizome has been studied in detail earlier, and the main constituents identified were ar-turmerone, turmerol and atlantone [10]. But the leaves of Curcuma species are a waste product during post-harvest operations. Traditionally, the leaves of *C. longa* are extensively used in culinary preparation, are aromatic and contain essential oil. There were no reports available regarding the composition and antioxidant potential of essential oil from curcuma longa leaf. The present study aims at an investigation of the major constituents and free radical scavenging activities of essential oil isolated from curcuma longa leaves.

2. Materials and methods

2.1. Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), gallic acid, ascorbic acid, linoleic acid, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) were purchased from Sigma-Aldrich (St. Louis MO, USA). Potassium ferricyanide, sodium phosphate monobasic, sodium phosphate dibasic, metaphosphoric acid was purchased from Merck (Germany). Trichloroacetic acid, ferric chloride, butylated hydroxyl toluene (BHT) was from Sisco Research laboratories (India). All other chemicals and solvents used were of standard analytical grade.

2.2. Plant material

The turmeric leaves (Alleppy variety) were collected from the local areas of Thiruvananthapuram district, Kerala, India authenticated and identified by taxonomist from Department of Botany, University of Kerala, India.

2.3. Isolation of essential oil from Curcuma longa leaves

The fresh leaves of turmeric were collected, washed, dried and powdered. 100 g of dried turmeric leaves powder was hydro distilled for 5 h in a Clevenger type apparatus. The oil was dried over anhydrous sodium sulphate and used for GC-MS analyses. The volatile oil percentage was obtained as 1.98%.

2.4. GC-MS analyses

GC-MS analyses were carried out in a Shimadzu GC-MS model GC-17A equipped with Mass spectrophotometer GC-MS QP 5050 A. A 30 M capillary silicon column was used for the analysis. Temperature programming conditions were as follows, temperature programming from 80 -200oC at the rate of 5oC per min and hold at 200oC for 25 min, injection temperature 250oC, interface temperature 270oC, carrier gas helium, flow rate of 1 mL/min, split ratio 1:50, and MS conditions were electron impact, ionizing voltage 70 eV, source temperature 150oC, electron multiplier at 2000 eV, scan speed 690 amu/s and scan range 40- 500 amu.

The retention indices of compounds were determined relative to the retention times of a series of n-alkanes with linear interpolation. Identification of the oil components was done by comparison of their mass spectra with the Wiley GC/MS library as well as by comparing them with those reported in literature. The identification of each compound was confirmed by comparison of its retention index either with those of authentic compounds or from literature.

2.5 Evaluation of antioxidant and free radical scavenging activity

2.5.1 DPPH Radical Scavenging activity

The free radical scavenging activity of the oil was determined using 1,1-diphenyl-2- picrylhydrazyl(DPPH) [11,12]. Briefly, 3 mL of extract was added to 1 mL of DPPH (1, 1-diphenyl-2-picrylhydrazyl) solution (0.2 mM in methanol) as the free radical source. The mixture was shaken and kept for 30 minutes at room temperature. The decrease in absorbance due to the proton donating activity of essential oil was determined at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Gallic acid was used as the positive control.

2.5.2 ABTS radical scavenging assay

The antioxidant activity was determined using an improved ABTS decolorisation assay [13] which involves the generation of $ABTS_+^{\cdot}$ chromophore by the oxidation of ABTS with potassium persulfate. The ABTS radical cation ($ABTS_+^{\cdot}$) was produced by reacting 7 mM stock solution of ABTS with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark for at least 6 h at room temperature before use. The $ABTS_+^{\cdot}$ solution was diluted to an absorbance of 0.7 ± 0.05 at 734 nm (Shimadzu UV-Vis spectrophotometer, Model 2100). Absorbance was measured 7 min after the initial mixing of different concentrations of the oil and the reference compound with 2.7 mL of $ABTS_+^{\cdot}$ solution. The $ABTS_+^{\cdot}$ scavenging capacity of the extracts were compared with that of trolox.

2.5.3. Total reducing power

The reducing power of oil was determined according to the method of Jayanthi *et al*[14]. Different concentrations of methanolic solutions of sample were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The mixture was incubated at 50 ℃ for 20 min. After 2.5 mL of TCA (10%) was added, the mixture was centrifuged at 3000 rpm for 10 min. Supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and 0.5 mL of ferric chloride (0.1%) and the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicates greater reducing power.

2.5.4.Antiperoxidative potential in linoliec acid emulsion system

The antioxidant activity of turmeric leaf essential oil was determined by the thiocyanate method[13]. Different concentrations of the sample in methanol were mixed with linoleic acid emulsion in potassium phosphate buffer (0.02 mol, pH 7.0) and the reaction mixture was incubated at 370C. Aliquots of 0.1mL were taken at various intervals during the incubation. The degree of oxidation was measured by sequentially by adding ethanol (5 mL, 75%), ammonium thiocyanate (0.1 mL, 30%) and ferrous chloride (0.1 mL, 0.02 mol in 3.5% HCl) to the sample solution (0.1 mL), and the absorbance

was read at 500 nm. Solutions without added extracts were used as blank samples.

3. Results

3.1. GC-MS

The chemical compositions of the oils with retention indices and% composition are given in the Table 1. Ninety eight percent of the compounds were identified in *Curcuma longa* leaf oil. β – sesquiphellandrene(22.8%) was the major compound, followed by terpinolene(9.5%), and aromatic curcumene (7.8%). Monoterpene compounds accounted for 30.9% and some of the major monoterpene hydrocarbons were terpinoline (9.5%), 1,8-Cineole (6.3%), α -Phellandrenene (4.8%), β -pinene (2.2%). The total sesqui terpenoids accounted for about 67.2%.The major component was β -sesquiphellandrene (22.8%) followed by aromatic-curcumene (7.8%), E-nerolidol (5.8%), Zingiberene (4.2%) and Cis-sesquisabinene hydrate (3.4%).

Table 1
Chemical composition of turmeric leaf essential oil.

Compound	KI	Content (%)
alpha-pinene	939	0.5
beta-pinene	980	2.2
beta-myrcene	991	1.3
Al-phellandrene	1004	4.8
d-3-carene	1009	0.6
1,8 cineole	1028	6.3
g-terpinene	1058	0.6
terpinolene	1080	9.5
linalool	1098	1.1
p-mentha,1,3,8,triene	1110	0.8
myrtenal	1163	0.5
a-terpineol	1187	1.7
Cis-sabinol	1189	0.5
myrtenol	1194	0.3
carvacrol	1290	0.2
b-elemene	1390	1.3
b-caryophyllene	1418	3.3
b-farnesene	1458	2.5
Ar-curcumene	1483	7.8
zingiberene	1490	4.2
b-sesquiphellandrene	1524	22.8
g-bisabolene	1530	1.3
Cis-sesquisabinene hydrate	1540	3.4
E-nerolidol	1564	5.8
Ar-turmerol	1576	3.1
Caryophyllene oxide	1580	1.8
curzerenone	1590	0.6
Ar-turmerone	1636	1.8
Al-turmerone	1642	1.6
b-bisabolol	1662	2.2
turmerone	1670	1.9
Farnesol(Z,Z)	1693	0.6
Farnesol(Z,E)	1699	1.2
Total		98.1

3.2. DPPH Radical Scavenging Assay

Figure 1 shows the DPPH radical scavenging activity of turmeric leaf essential oil. Various concentrations of the oil was evaluated for their DPPH radical scavenging activity. The estimated IC_{50} values for the oil were found to be 3.227 mg/mL and that of standard compound gallic acid is 1.32 μg/mL and is inversely related to antioxidant capacity.

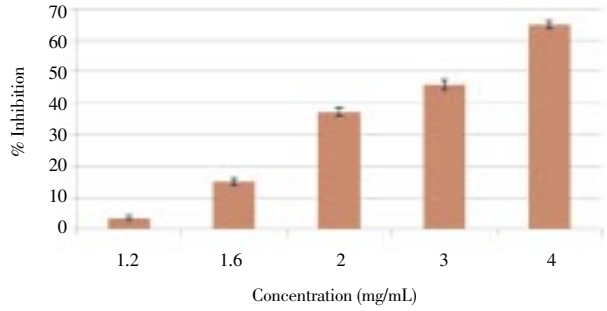

Figure 1. DPPH Radical scavenging activity of turmeric leaf oil.
Each value is expressed as mean ± SD of triplicate measurements and the significance accepted at $P < 0.05$.

3.3. ABTS Radical scavenging assay.

Figure 2 shows a steady increase in the ABTS radical scavenging capacity of turmeric leaf oil. The estimated IC50 values were found to be 1.541 mg/mL for oil and that of the standard compound, trolox was 2.27 μg/mL.

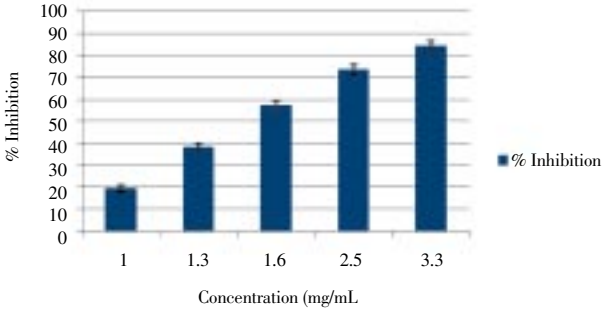

Figure 2. ABTS radical scavenging activity of turmeric leaf oil.
Each value is expressed as mean ± SD of triplicate measurements and the significance accepted at $P < 0.05$.

3.4. Total reducing power (TRP)

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity[15]. Reducing power is to the measure of the reductive ability of antioxidant and it is evaluated by the transformation of Fe^{3+} to Fe^{2+} in the presence of sample[16]. The total reducing power

(TRP) of oil of turmeric leaves was evaluated and compared with the standard compounds like Gallic Acid, ascorbic acid and BHA (Figure 3). The TRP observed in the present study was found to be Gallic acid (GA)> Ascorbic acid (AA)> BHT> turmeric leaf oil.

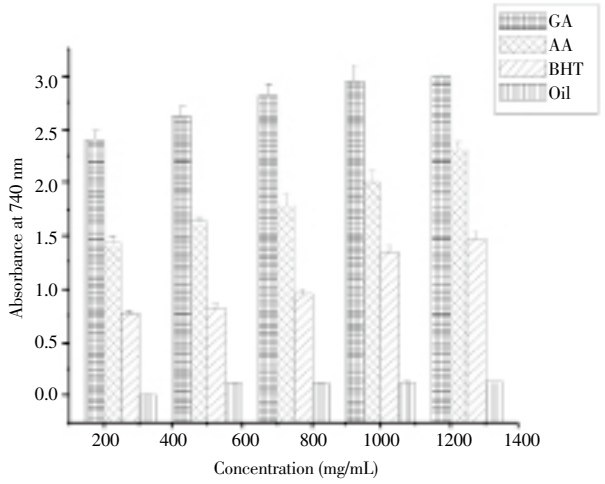

Figure 3. Total reducing power (TRP).
Each value is expressed as mean ± SD of triplicate measurements and the significance accepted at $P < 0.05$.

3.5. Antiperoxidative potential in linoleic acid emulsion system

Antiperoxidative potential of turmeric leaf oil was evaluated using linoliec acid emulsion system and was compared with that of standard compound BHT (Figure 4). Turmeric oil inhibited linoleic acid peroxidation (72.51%) at a concentration of 1 mg/mL while that of BHT inhibited peroxidation (82.71%) at a concentration of 100 μg/mL.

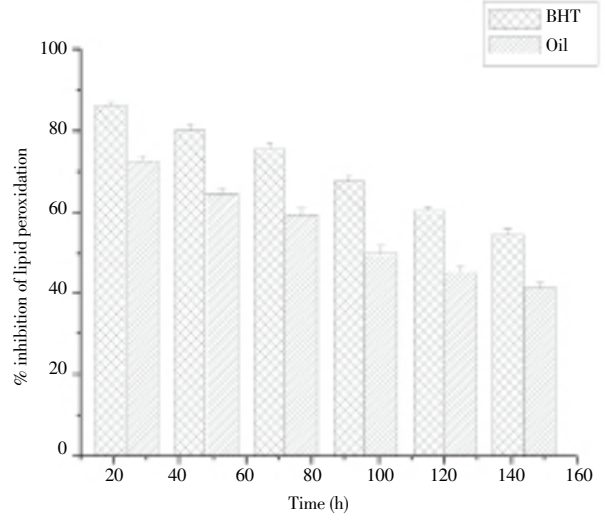

Figure 4. Antiperoxidative potential of turmeric leaf essential oil in linoleic acid emulsion system.
Each value is expressed as mean ± SD of triplicate measurements and the significance accepted at $P < 0.05$.

4. Discussion

Thirty five compounds representing 98.1% of leaf oil were identified. However, to the best of our knowledge, there have been no reports on chemical composition of turmeric leaf oil from alleppy variety and this is the first report regarding the chemical composition of the turmeric oil. The antioxidant potential of an essential oil can be mainly attributed to its reducing power and its radical scavenging ability[7].

The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability[13]. DPPH, a stable nitrogen centred free radical, has been used to evaluate natural antioxidants for their radical quenching capacities in a relatively short time, compared with other methods [9]. The method is based on the reduction of the absorbance of methanolic DPPH solution at 517 nm in the presence of proton donating substance due to the formation of the diamagnetic molecule by accepting an electron or hydrogen radical[9,13]. The result shows that turmeric leaf oil can act as a proton donor and an antioxidant.

The relative antioxidant ability to scavenge the radical has been compared to the standards Trolox and gallic acid and is an excellent tool for determining the antioxidant activity of hydrogen donating antioxidants and of chain breaking antioxidants [13]. This method is applicable both for lipophilic and hydrophilic substances. The results revealed that turmeric leaf oil can scavenge $ABTS_{+}$• in a dose dependant manner and can act as hydrogen donating antioxidant or chain breaking antioxidant. The antioxidant activity of a substance is also supported by its reducing power. The reducing power of the turmeric leaf oil and the reference compounds ascorbic acid, gallic acid and BHA increased steadily with the increasing concentration. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant activity by breaking the free radical chain by donating a hydrogen atom[13]. Antiperoxidative potential of turmeric leaf oil was evaluated using linoliec acid emulsion system. Oxidation of linoleic acid produces hydroperoxides, which decomposes to many secondary oxidation products. These oxidized products react with ferrous chloride to formferric chloride which on, further reaction with ammonium thiocyanate, forms the ferric thiocyanate red color. Antioxidants can slow down the peroxidation of linoleic acid; hence the ferric thiocyanate formation will be slow. The linoelic acid emulsion system can be simulated with the biological lipid system or with food/fat emulsions[9]. The data suggests that as a natural antioxidant, turmeric leaf oil can be used to prevent the oxidative deterioration of fat containing food systems or reactive oxygen species mediated tissue damage in biological systems.

In conclusion, results of the present study reveals that

essential oil isolated from curcuma leaf may serve as an antioxidant and can be used as a potent candidate for the prevention of oxidative stress mediated damages.

Conflict of interest statement

We declare that we have no conflict of interest.

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